

# PATENT ABSTRACTS OF JAPAN

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(54) APPARATUS FOR CULTURING PHOTOSYNTHETIC MICROORGANISM  
AND CULTURING METHOD

(57)Abstract:

PROBLEM TO BE SOLVED: To provide an apparatus for culturing photosynthetic microorganism and a culturing method enabling the industrial utilization of photosynthetic microorganisms by the improvement of photo-utilization efficiency and the attainment of a high-density culture.

SOLUTION: Both side faces of this photosynthetic microorganism culturing apparatus are made of a light-transmitting transparent material such as transparent plastic or transparent glass and the apparatus is lighted with lighting means 30 placed at both sides 11 of the apparatus. The thickness of the culture tank 1 is set to about  $\leq 2$ cm. An unexpected increase of productivity unexplainable by the increase of light quantity is attained by decreasing the thickness to  $\leq 2$ cm.

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## **CLAIMS**

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### **[Claim(s)]**

**[Claim 1]** The photosynthesis bacterial culture apparatus characterized by having an optical supply means by which a both-sides side is made from light transmission nature transparent materials, such as a transparent plastic or clear glass, at least, and light was supplied by the cultivation tank whose distance between these both-sides sides is about 2cm or less, and its side face.

**[Claim 2]** The photosynthesis bacterial culture apparatus according to claim 1 characterized by having further the constant temperature bath which holds said transparency cultivation tank selectively at least.

**[Claim 3]** An optical supply means is a photosynthesis bacterial culture apparatus according to claim 1 characterized by being what uses artificial sources, such as a fluorescent lamp, an incandescent lamp, and a xenon lamp, as the light source.

**[Claim 4]** The photosynthesis bacterial culture apparatus according to claim 1 characterized by enabling accommodation of the distance of said light source and cultivation tank.

**[Claim 5]** Said cultivation tank is a photosynthesis bacterial culture apparatus according to claim 1 characterized by having an aerator further, carrying out aeration of air or the air which added the carbon dioxide several% artificially 1l. [ per 1l. culture medium ] or more in 1 minute from there, and enabling stirring of culture medium with the bubble which goes up the inside of culture medium.

**[Claim 6]** The photosynthesis bacterial culture apparatus according to claim 1

characterized by having the swelling section for pH electrode insertion further in some cultivation tanks.

[Claim 7] Said cultivation tank is a photosynthesis bacterial culture apparatus according to claim 1 characterized by having in a tub the baffle which inclined aslant [ aiming at the improvement in structure on the strength of a cultivation tank, and the improvement in stirring effectiveness of culture medium ].

[Claim 8] claim 1 thru/or 7 -- the culture approach of the photosynthesis microorganism which is the approach of cultivating a photosynthesis microorganism using the photosynthesis bacterial culture apparatus indicated to either, and was characterized by supplying an always required nutrition and removing discard by performing solid liquid separation, reflux to the cultivation tank of a cell, and makeup of a fresh culture medium continuously or semi-continuously.

[Claim 9] claim 1 thru/or 7 -- the culture approach of the photosynthesis microorganism characterized by to perform optical heterotrophy-culture of the hybrid model of the optical autotrophy which is the approach of cultivating a photosynthesis microorganism using the photosynthesis bacterial culture apparatus indicated to either, and added other organic and inorganic carbon sources in addition to the optical autotrophy-culture which uses as the only carbon the carbon dioxide or the carbon dioxide added auxiliary in air, and a heterotrophy.

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## DETAILED DESCRIPTION

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### [Detailed Description of the Invention]

#### [0001]

[Field of the Invention] This invention relates to the culture apparatus and the culture approach of a photosynthesis microorganism which enabled efficient culture by using thin equipment for a detail further about the culture apparatus and the culture approach of a photosynthesis microorganism.

#### [0002]

[Description of the Prior Art] The microorganism (for example, detailed algae, such as green algae and blue-green algae, a photosynthetic bacterium) increased in optical autotrophy compounds the organic substance from a carbon dioxide and water using luminous energy, and is leading the life. Therefore, in order to cultivate a photosynthesis microorganism efficient, it becomes the important point how light is supplied efficiently. The culture apparatus which used the side-face luminescence mold optical fiber about this point until now, The culture apparatus which applied a perimeter to light to the jar fermenter for some bacterial culture from the former, Various configurations, such as a culture apparatus, a tube mold culture apparatus, etc. which protected the fluorescent lamp with the glass tube and were installed in the tub, The culture apparatus of a format is proposed (utilization and actual" of the artificial light in a Kazunori Fujita "bioreactor, the foundation for the world-practice person of a bioreactor and HARIO lab "application [ - ]" 1992 issuance).

#### [0003]

[Problem(s) to be Solved by the Invention] However, it has the problems that any culture apparatus by which the conventional proposal is made has an upper limit in the optical reinforcement of luminescent material with the high cost of an

ingredient, like that equipment is complicated and a scale-up is difficult. Moreover, it has not resulted in what also not necessarily satisfies yield. This invention aims at offering the photosynthesis bacterial culture apparatus and the culture approach of attaining the high cell concentration which improves improvement in the utilization effectiveness of light energy, and a proliferation rate, and could not reach by the conventional approach simple while it cancels the above inconvenience of the conventional photosynthesis bacterial culture apparatus.

[0004]

[Means for Solving the Problem] As a result of repeating an experiment and research wholeheartedly about the above-mentioned technical problem, by making thickness of the cultivation tank extremely thin (about 2cm or less) in the photosynthesis bacterial culture apparatus of the core box proposed until now, this invention person etc. perceives that the unexpected high productivity which cannot be explained only by the increment in the quantity of light is attained, and came to make this invention.

[0005] That is, the photosynthesis bacterial culture apparatus of this invention is characterized by having an optical supply means by which a both-sides side is made with a transparent plastic or clear glass at least, and light was supplied by the transparency cultivation tank whose distance between these both-sides sides is 2cm or less, and its side face.

[0006] in order to control the effect of the heat from the light source, or this cultivation tank is preferably installed into the transparency constant temperature bath for temperature control -- or the surroundings of a cultivation tank -- constant temperature -- the transparent jacket for water cycles is prepared. Moreover, you may make it irradiate light if possible from one side or the both-sides side of a cultivation tank at homogeneity, or may make it make it photosynthesize efficiently to detailed algae etc. using natural sunlight, using artificial sources, such as a fluorescent lamp, an incandescent lamp, or a xenon lamp, as the light source. It is a desirable mode, and by it, the accommodation of the optical reinforcement inside a cultivation tank also of also enabling

accommodation of the light source and the distance of a cultivation tank can be attained, and it can raise efficiency for light utilization.

[0007] Since it is stirring of culture medium, and in order to attain complete-mixing conditions, it is an especially desirable mode to install a tube-like aerator in a cultivation tank pars basilaris ossis occipitalis. From this aerator preferably aeration of air or the air which added the carbon dioxide several% artificially 1l. [ per 1l. culture medium ] or more is carried out in 1 minute, and culture medium is stirred with the bubble which goes up the inside of culture medium -- cultivating [ of high density ] becomes rattlingly alike much more possible.

[0008] Moreover, in order to maintain optimal pH conditions which change with various photosynthesis microorganisms, it is a desirable mode to measure pH continuously and to perform pH control with the pH electrode which gave the swelling for pH electrode insertion to some cultivation tanks, and was inserted in it. Furthermore, installation into the tub of the baffle which inclined aslant for a while is also good better \*\*\*\*\*, and becomes possible [ attaining three objects called improvement in the speed of response at the time of being the improvement in structure reinforcement of the thin cultivation tank whose distance between both-sides sides is 2cm or less, improvement in the stirring effectiveness of culture medium, and pH control ] by installing such a baffle.

[0009] The culture approach using the photosynthesis bacterial culture apparatus and it by this invention is \*\* in which culture by the high density which was not able to be expected to the former is possible by taking the culture approach of performing makeup of an always required nutrient, and clearance of discard by doing solid liquid separation, reflux to the cultivation tank of a cell, and fresh culture-medium supply simultaneously continuously or semi-continuously, although high density cultivation is suitable if a deployment of the installation tooth space of equipment and the ease at the time of photosynthesis microbial cell harvest are taken into consideration.

[0010] As a photosynthesis microorganism cultivated using the equipment of this invention, the detailed algae of the shape of a photosynthetic bacterium,

unicellular detailed algae, and yarn, unicellular blue-green algae, yarn-like blue-green algae, etc. are mentioned as an object. Moreover, a certain \*\*\*\* culture medium used for detailed algae as a culture medium used for these culture until now is usable. As an example, they are BG-11, MC, ESM, PES, SOT, MDM, multiple buoy mooring, etc. moreover, the light which added carbon sources other than a carbon dioxide to the culture medium -- subordinate culture is also possible.

[0011] As construction material of a culture apparatus, transparency, such as an acrylic, glass, and a polycarbonate, is high, and if there is no cytotoxicity, all are possible, but when premised on an outdoor activity, it is effective to apply an ultraviolet absorption film to a material-list side. Although the magnitude of equipment requires that the thickness of cultivation tank inside distance should be 2cm or less, based on a capacity required for culture, height and width of face can be freely designed except it.

[0012] Moreover, if the temperature in a cultivation tank is controllable to homogeneity, especially the size of the transparence cistern for temperature control will not be restricted, either. As mentioned above, especially a limit does not have the light source, either and especially a limit does not have optical reinforcement, either. In order to maintain sufficient stirring effectiveness, 1l. or more per 1l. culture medium of quantity of airflow is desirable in at least 1 minute. The gas which carries out aeration has the desirable mixed gas which added about several% of carbon dioxide to air. As a cellular generator, a glass sparger etc. is unnecessary, in a tube with a bore [ made from stainless steel ] of about 5mm, every other cm, it is an open beam thing and the object can fully attain an about 1mm hole. Although especially the upper part of a cultivation tank can also be opened by not covering, it is also possible to seal by covering, in performing sterile culture, to introduce 5-10% of hydrogen peroxide in equipment, and to sterilize.

[0013]

[Embodiment of the Invention] Hereafter, the gestalt of desirable operation of the

photosynthesis bacterial culture apparatus by this invention is explained with reference to a drawing. constant temperature for 1 to adjust the body of a cultivation tank and for 2 adjust [ drawing 1 is the decomposition perspective view showing an example of the photosynthesis bacterial culture apparatus by this invention, ] the temperature of the body of a cultivation tank in drawing, -- it is a transparency jacket for water cycles. The body 1 of a cultivation tank has the shape of a core box thin as a whole, and the both-sides sides 11 and 11 are made from the transparent material in which light transmission, such as a transparent plastic or clear glass, is possible. And distance between the inner surfaces of the both-sides sides 11 and 11 which counter (namely, thickness of a cultivation tank) is set to 2cm or less. One flank of the body 1 of a cultivation tank is the swelling section 12 made a little broad, and the upper part of the body 1 of a cultivation tank including this swelling section is opened.

[0014] the constant temperature from the constant temperature bath which the lower half of the body 1 of a cultivation tank is held in the jacket 2, and is not illustrated in this jacket 2 in this operation gestalt -- it is introduced in a jacket 2, and water is drawn out and he is trying to circulate from the tap hole 22 prepared up from the input 21 prepared near the pars basilaris ossis occipitalis of a jacket 2 The tube 3 for aeration is formed near the pars basilaris ossis occipitalis of the body 1 of a cultivation tank, and this tube 3 for aeration is connected to the tube 31 (refer to drawing 2 ) for gassing arranged at the pars basilaris ossis occipitalis of the body 1 of a cultivation tank. It has connected with the supplied-air pump which is not illustrated, air or air+2 carbon-monoxide mixed gas is supplied through a supplied-air pump, and the tube 3 for aeration is emitted to culture medium as air bubbles from the tube 31 for gassing within the body 1 of a cultivation tank.

[0015] The upper part clear aperture 13 of the body 1 of a cultivation tank is closed by the silicon rubber seal 4 for sealing, and the lid 5 in seal. The opening 8 for an air vent and a sampling is formed in the part which anchoring of the thermo sensor which is not illustrated, pH sensor, etc. and the opening 6 for acid

alkali addition are formed in the part equivalent to the swelling section 12 of the body 1 of a cultivation tank of a lid 5 and the silicon rubber seal 4 for sealing, and is equivalent to the body 1 of a cultivation tank. A lid 5 and the silicon rubber seal 4 for sealing are bound tight with the proper clamp 7, and ensure sealing. In addition, when performing culture in the open condition, the silicon rubber seal 4 for sealing, a lid 5, a clamp 7, etc. are not used.

[0016] The optical supply means for [ of the body 1 of a cultivation tank ] supplying light to the culture medium in a cultivation tank in a side-face location is arranged at least. In the example of drawing 1, the fluorescent lamp group 30 of a large number close to the both-sides sides 11 and 11 of the body 1 of a cultivation tank considers as an optical supply means, and is arranged. As described above, the light source can use artificial sources, such as not only a fluorescent lamp but an incandescent lamp, a xenon lamp, etc., for arbitration, and may use natural sunlight. Although not illustrated, it becomes possible by enabling accommodation of the light source and the distance of a cultivation tank using a proper migration means to make the photosynthesis conditions suitable for many culture modes.

[0017] The bow baffle 14 which drawing 2 is the side elevation of the cultivation tank shown in drawing 1, and inclined for a while is installed in the tub. The bow baffle 14 is made into the condition of having lost preferably touch with a bottom of the tank side a little, and is arranged fixed by the proper fixed means among the both-sides sides 11 and 11. In the stirring effectiveness to the longitudinal direction of the improvement in on the strength of a cultivation tank, and the culture medium in a tub, this baffle is formed in order to aim at response-time compaction of raising pH and temperature control, and number of sheets etc. is set up whenever [ optimal tilt-angle ] experimentally. In addition, by drawing 2, an arrow head arranges the bow baffle 14 in the gestalt of a graphic display, and sketches the example of a position of the flow of the culture medium at the time of sending air in a cultivation tank through the tube 31 for gassing from the tube 3 for gas aeration from a side face. In addition, in drawing 2, the thermo sensor S1

and the pH sensor S2 are attached in the opening 6 formed in the lid 5.

[0018] In addition, having been shown in drawing 1 and 2 does not pass to an example of the photosynthesis bacterial culture apparatus by this invention, but it may have many deformation. For example, although the tube 31 for gassing shows only one to the pars basilaris ossis occipitalis of equipment, it changes height and you may make it install it. [ two or more ] Moreover, you may make it insert the tube 3 for aeration through the opening 6 prepared in the swelling part of said lid 5. It may not be indispensable to curve, as the configuration of a baffle was also shown in drawing 2 , and it may be a straight-line-like thing. Moreover, if the thickness of the body 1 of a cultivation tank removes the limit of 2cm or less, especially a limit will not have the configuration of a cultivation tank, and a ratio in every direction, either.

[0019] To the photosynthesis bacterial culture apparatus pan, drawing 3 is other operation gestalten and is juxtaposing the fluorescent lamp group 30 and cultivation tank as the light source by turns in this example. In drawing, 41 is a carbon-dioxide bomb, 42 is a compressor for air aeration, and a carbon dioxide and air are made into a desired mixing ratio by the gas blender 43, and are sent in in a cultivation tank through the tube 3 for gas aeration. Moreover, in drawing, 44 is a duct for culture medium recovery, and can collect culture medium through this duct 44. Thus, amplification of culture magnitude can be easily aimed at by arranging two or more cultivation tanks in juxtaposition.

[0020]

[Example] Hereafter, an example explains this invention concretely.

(Example 1) The culture apparatus of this invention is used and it is marine green algae *Chlorococcum littorale*. The cultivated example is shown in drawing 4 . What added the following nutritive salts to 1l. of seawater of 1/2 concentration which mixed the seawater extracted from the Iwate Kamaishi bay and tap water by 1 to 1 was used as culture medium.

[0021]

KNO<sub>3</sub> 2.5 gMgSO<sub>4</sub> and 7H<sub>2</sub>O 1.25 gKH<sub>2</sub>PO<sub>4</sub> 1.25 gFeSO<sub>4</sub> and 7H<sub>2</sub>O 2.2

mgH<sub>3</sub>BO<sub>3</sub> 0.72 mgMnSO<sub>4</sub> and 7H<sub>2</sub>O 0.63 mgZnSO<sub>4</sub> and 7H<sub>2</sub>O 0.056 mgCuSO<sub>4</sub> and 5H<sub>2</sub>O 0.020 mgNaMoO<sub>4</sub> 0.0053mg [0022] Only the concentration of the nitrate of MC culture medium doubles this presentation. The detailed alga of this \*\* was planted in this culture medium, by 71-/min, aeration of the air containing 2 - 3% of carbon dioxide was carried out, and it was stirred. Temperature was kept at 25 degrees C and pH was not controlled. After having dried the filter at 105 degrees C after carrying out attraction filtration of the culture medium of optimum dose, collecting cells on the filter made from glass fiber (Whatman, GF/C) and 3 more% of ammonium formate's washing, and reaching constant weight, weighing capacity of the dry weight was carried out, and it was found. When continuous irradiation of the white fluorescent lamp was carried out from both sides for 24 hours and average light reinforcement was set to 1500micromol/m<sup>2</sup> / s, the straight-line term proliferation rate of 8.1 gdycell/l/d was obtained with the cultivation tank of 1cm thickness. This value is twice [ at least / more than ] the proliferation rate of the detailed algae cultivated by the optical autotrophy reported until now.

[0023] Changing only the thickness of a cultivation tank into 2cm and 4cm, others cultivated on the same conditions. Consequently, the proliferation profile shown in drawing 4 was obtained. When the thickness of a cultivation tank increased 4 times (4cm), the straight-line term proliferation rate was set to one eighth. This has suggested that detailed algae can be cultivated more efficiently [ as the thickness of a reaction vessel is small ].

[0024] That is, if thickness of a cultivation tank is made thin with 4cm, 2cm, and 1cm, as shown in drawing 4 , productivity (straight-line term proliferation rate) will rise with 1, 4, and 8 g/l/d. The amount of culture medium will be set to /1/4 by 1 / 2 or 1cm by 2cm, if the case of 4cm thickness is set to 1. The area which receives light is fixed, and a light-receiving interview / volume will be set to 2 and 4 if the case of 4cm is set to 1. That is, the amount of the light received in per volume of culture medium becomes 2 or 4 times. Although it was predicted that productivity is proportional to light income and productivity also increased 2 or 4

times therefore when following the conventional idea, actually, it had become 4 or 8 times and this was the result of the ability not expecting.

[0025] Although the light energy per volume has only doubled in 4->2cm, by 2->1cm, productivity is twice as expected to productivity being 4 times. That is, in thickness reduction of a 4->2cm cultivation tank, it can say that the qualitative shift which cannot be explained only by reducing thickness has happened, and it is proved that it is significant that the distance between the both-sides sides of a cultivation tank in this invention is about 2cm or less. In addition, it is thought with a 1cm cultivation tank that it is based on an exhaustion of nutrient salt (especially nitrogen source) after the 20th hour that the inclination of a proliferation profile is decreasing.

[0026] (Example 2) By using this equipment, it is marine green algae *Chlorococcum littorale* to high density, so that it was not able to attain in a conventional method. The cultivated example is shown in drawing 5 . As a result of performing culture-medium exchange of harvesting a cell according to centrifugal separation with a suitable time interval, moving to a fresh culture medium, and cultivating with this equipment again, the maximum cell concentration reached 84 gdrycell/l.

[0027] The arrow head in drawing shows the event of performing culture-medium exchange. This Tokimitsu reinforcement is 2300micromol/m<sup>2</sup> / s, and uses the cultivation tank of 1cm thickness. That which doubled [ further ] the concentration of the nitrate of the culture medium of an example 1 (5 g/l) was used for the culture medium. using the culture apparatus of this invention, although the value called 50 - 60 gdrycell/l by the heterotrophy-culture (namely, culture which added carbon sources other than a carbon dioxide in the culture medium) using chlorella etc. is reported conventionally -- moreover, it became possible by using the culture approach of this invention to cultivate detailed algae to high concentration further.

[0028]

[Effect of the Invention] This invention offers the new culture apparatus and new

approach of a photosynthesis microorganism. Although it was thought that culture of optical autotrophic microorganisms was inferior to a heterotrophy microorganism in respect of cost and a rate, it becomes possible to reclaim the new aspect of affairs of industrial utilization of a photosynthesis microorganism by the improvement in efficiency for light utilization and the achievement of high density cultivation which were obtained as a result of this invention.

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#### DESCRIPTION OF DRAWINGS

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[Brief Description of the Drawings]

[Drawing 1] The decomposition perspective view showing 1 operation gestalt of the photosynthesis bacterial culture apparatus by this invention.

[Drawing 2] The side elevation of the equipment shown in drawing 1 . The flow of culture medium is also shown.

[Drawing 3] The perspective view explaining the condition of having arranged main culture equipment and an optical feeder in juxtaposition, and having expanded culture magnitude.

[Drawing 4] Main culture equipment is used and it is marine green algae Chlorococcum littorale. Graph which shows the cultivated result.

[Drawing 5] The graph which shows the result of having repeated culture-medium exchange and having carried out high density cultivation of marine green algae *Chlorococcum littorale* using main culture equipment.

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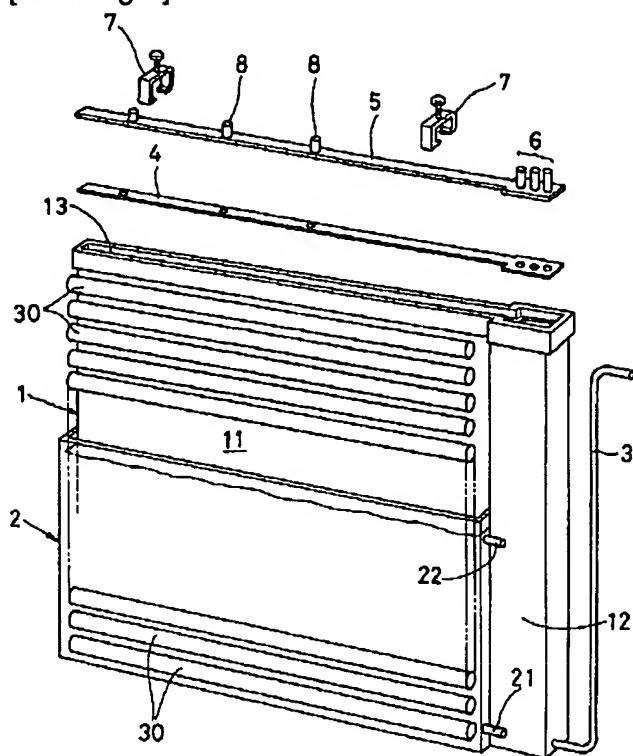
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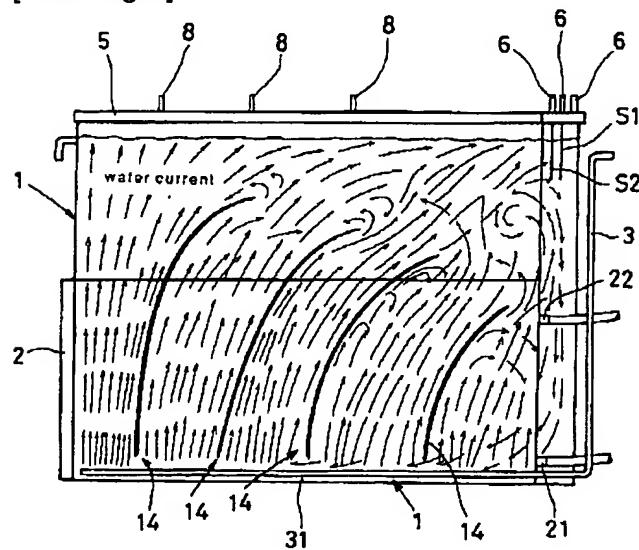
DRAWINGS

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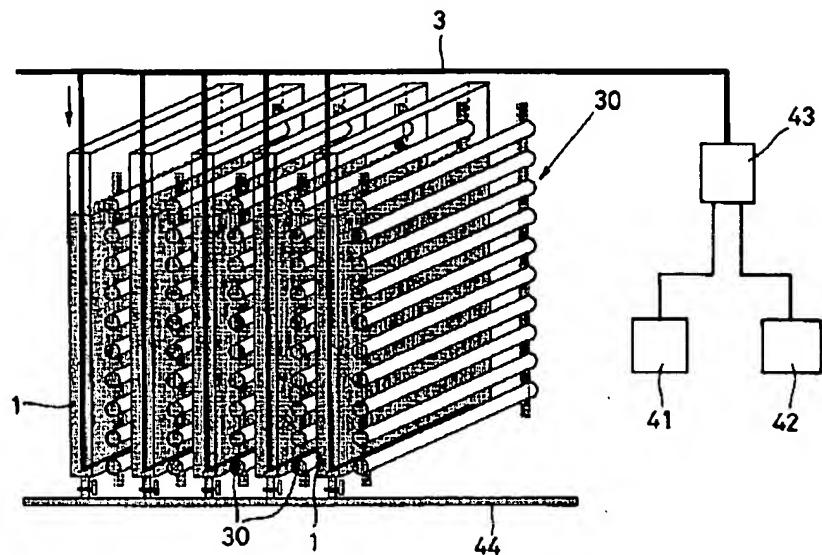
[Drawing 1]



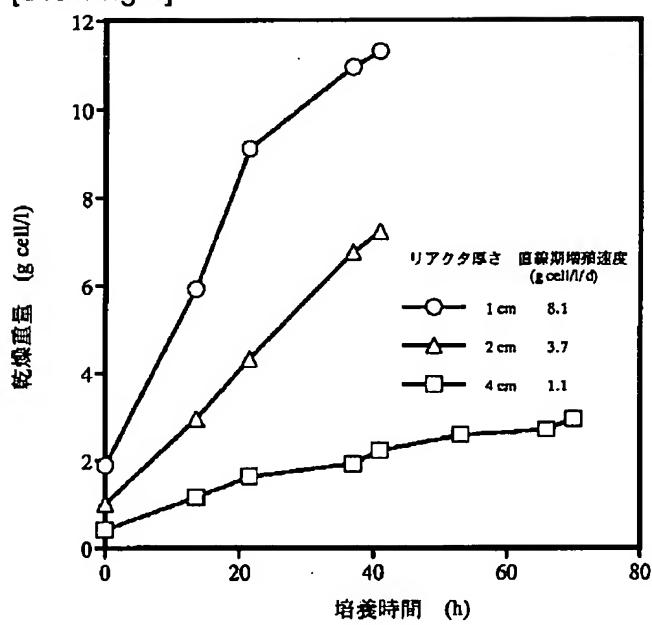
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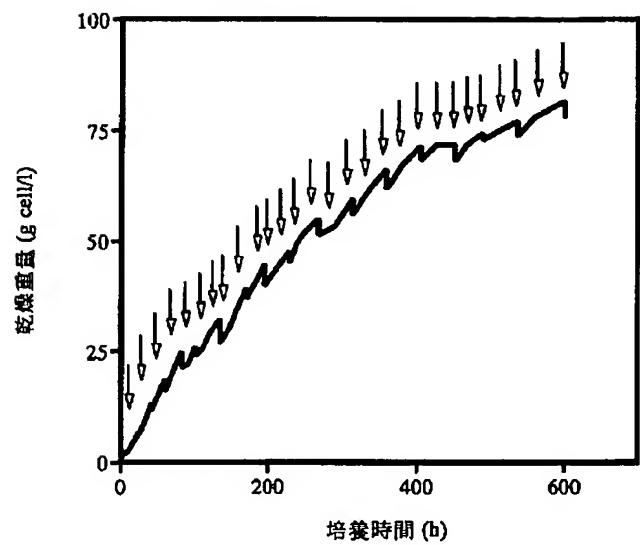
[Drawing 3]



[Drawing 4]



[Drawing 5]



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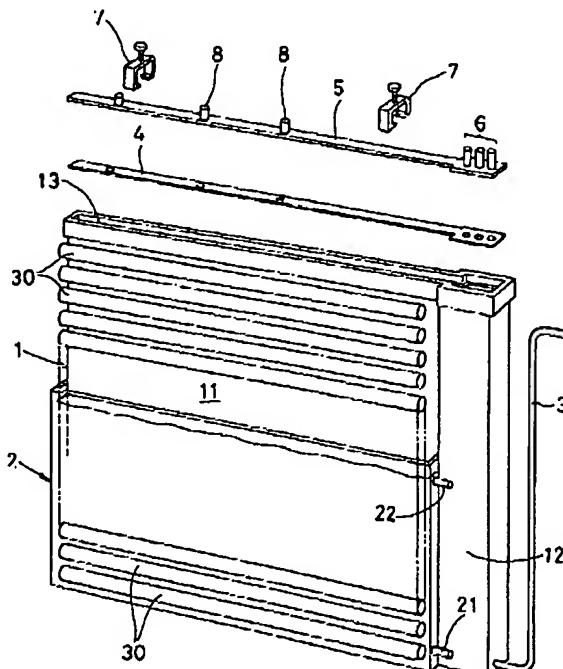
(74)代理人 弁理士 平木 祐輔 (外1名)

(54)【発明の名称】 光合成微生物培養装置及び培養方法

(57)【要約】

【課題】 光利用効率の向上と高密度培養の達成によって、光合成微生物の産業的利用を可能とする光合成微生物培養装置及び培養方法を提供する。

【解決手段】 両側面が透明プラスチックあるいは透明ガラスなどの光透過性透明材料で作られ、両側面に配置した光供給手段から光を供給するようにした光合成微生物培養装置において、培養槽の厚みをほぼ2cm以下とする。厚みを2cm以下としたことにより、光量の増加では説明できない、予期せぬ生産性の増大がもたらされた。



## 【特許請求の範囲】

【請求項1】 少なくとも両側面が透明プラスチックあるいは透明ガラスなどの光透過性透明材料で作られかつ該両側面間の距離がほぼ2cm以下である培養槽とその側面に光を供給するようにされた光供給手段とを有することを特徴とする光合成微生物培養装置。

【請求項2】 前記透明培養槽を少なくとも部分的に収容する恒温水槽をさらに有することを特徴とする請求項1に記載の光合成微生物培養装置。

【請求項3】 光供給手段は、蛍光灯、白熱電球、キセノンランプなどの人工光源を光源として用いるものであることを特徴とする請求項1に記載の光合成微生物培養装置。

【請求項4】 前記光源と培養槽の距離が調節可能とされていることを特徴とする請求項1に記載の光合成微生物培養装置。

【請求項5】 前記培養槽は通気装置をさらに有し、そこから1分間に1リッターの培養液あたり1リッター以上の空気あるいは人為的に二酸化炭素を数%付加した空気を通気して、培養液中を上昇する泡によって培養液を攪拌可能とされていることを特徴とする請求項1に記載の光合成微生物培養装置。

【請求項6】 培養槽の一部にpH電極挿入用の膨出部をさらに有することを特徴とする請求項1に記載の光合成微生物培養装置。

【請求項7】 前記培養槽は培養槽の構造強度向上と培養液の攪拌効率向上を目的とした斜めに傾いた邪魔板を槽内に有することを特徴とする請求項1に記載の光合成微生物培養装置。

【請求項8】 請求項1ないし7いずれかに記載した光合成微生物培養装置を利用して光合成微生物を培養する方法であって、連続的あるいは半連続的に固液分離と、細胞の培養槽への還流と、新鮮培地の補給を行うことによって常に必要な栄養の補給を行いつつ不要物の除去を行うことを特徴とした光合成微生物の培養方法。

【請求項9】 請求項1ないし7いずれかに記載した光合成微生物培養装置を利用して光合成微生物を培養する方法であって、空気中の二酸化炭素あるいは補助的に付加した二酸化炭素を唯一の炭素とする光独立栄養的な培養に加え、他の有機・無機の炭素源を添加した光独立栄養と從属栄養の混合型の光從属栄養的な培養を行うことを特徴とする光合成微生物の培養方法。

## 【発明の詳細な説明】

## 【0001】

【発明の属する技術分野】本発明は光合成微生物の培養装置および培養方法に関し、さらに詳細には薄型の装置を用いることによって高効率の培養を可能とした光合成微生物の培養装置および培養方法に関する。

## 【0002】

【従来の技術】光独立栄養的に増殖する微生物（例え

ば、緑藻やラン藻などの微細藻類、光合成細菌）は、光のエネルギーを利用して二酸化炭素と水から有機物を合成して生命を営んでいる。従って、高効率に光合成微生物を培養するためには如何に効率よく光を供給するかが重要なポイントとなる。この点に関して、これまでに側面発光型光ファイバを利用した培養装置、従来からある細菌培養用のジャーファーメンタに周囲から光を当てた培養装置、蛍光灯をガラス管で保護して槽内に設置した培養装置、チューブ型培養装置など種々の形状、様式の培養装置が提案されている（藤田一紀「バイオリアクターにおける人工光の利用とその実際」、ハリオ研究所「バイオリアクターの世界—実践者のためのその基礎と応用—」1992年発行）。

## 【0003】

【発明が解決しようとする課題】しかし、従来提案されているいずれの培養装置も、材料のコストが高い、発光材料の光強度に上限がある、装置が複雑化する、スケールアップが難しい等の問題を抱えている。また、収率も必ずしも満足するものには至っていない。本発明は、従来の光合成微生物培養装置の上記のような不都合を解消すると共に、光エネルギーの利用効率の向上と増殖速度を改善して従来の方法では達し得なかった高細胞濃度を簡便に達成することのできる光合成微生物培養装置及び培養方法を提供することを目的とする。

## 【0004】

【課題を解決するための手段】本発明者等は、上記の課題に関して鋭意実験と研究を重ねた結果、これまでに提案された箱型の光合成微生物培養装置において、その培養槽の厚みを極端に薄く（ほぼ2cm以下）することにより、単に光量の増加のみでは説明できない、予期せざる高い生産性が達成されることを知覚し、本発明をなすに至った。

【0005】すなわち、本発明の光合成微生物培養装置は、少なくとも両側面が透明プラスチックあるいは透明ガラスで作られかつ該両側面間の距離が2cm以下である透明培養槽とその側面に光を供給するようにされた光供給手段とを有することを特徴とする。

【0006】好ましくは、この培養槽は、光源からの熱の影響を抑制するために温度調節用の透明恒温水槽の中に設置されるか、あるいは培養槽の回りに恒温水循環用の透明なジャケットを設ける。また、光源として蛍光灯または白熱電球またはキセノンランプなどの人工光源を用い、培養槽の片面あるいは両側面からなるべく均一に光を照射するようにもよく、あるいは自然の太陽光を利用して微細藻類などに効率よく光合成を行わせるようにもよい。光源と培養槽の距離を調節可能とすることも好ましい態様であり、それによって、培養槽内部の光強度の調節が可能となり光利用効率を向上させることができる。

【0007】培養液の攪拌のために、また、完全混合条件

件を達成するために、培養槽底部にチューブ状通気装置を設置することは特に好ましい態様であり、該通気装置から、好ましくは、1分間に1リッターの培養液あたり1リッター以上の空気あるいは人為的に二酸化炭素を数%付加した空気を通気し、培養液中を上昇する泡によって培養液を攪拌することによって、一層高密度の培養が可能となる。

【0008】また、種々の光合成微生物によって異なる最適のpH条件を維持するために、培養槽の一部にpH電極挿入用の膨らみを持たせ、挿入したpH電極によってpHを連続測定し、pH制御を行うことは望ましい態様である。さらに、少し斜めに傾いた邪魔板の槽内への設置も好ました態様であり、このような邪魔板を設置することにより、両側面間の距離が2cm以下である薄型の培養槽の構造強度の向上、培養液の攪拌効率の向上、pH制御の際の応答速度の向上と言う3つの目的を達成することが可能となる。

【0009】本発明による光合成微生物培養装置及びそれを用いた培養方法は、装置の設置スペースの有効利用や光合成微生物細胞収穫時の容易さを考慮すると高密度培養が適しているが、連続的あるいは半連続的に固液分離と細胞の培養槽への還流と新鮮培地供給を併行することにより常に必要な栄養素の補給と不要物の除去を行う培養方法を取ることにより、従来には予期できなかった高密度での培養が可能となる。

【0010】本発明の装置を用いて培養する光合成微生物としては、光合成細菌、単細胞の微細藻類、糸状の微細藻類、単細胞ラン藻、糸状のラン藻などが対象として挙げられる。また、これらの培養に用いる培地としてはこれまでに微細藻類用に利用してきたあるゆる培地が使用可能である。具体例としては、BG-11、MC、ESM、PES、SOT、MDM、MBM等である。また、培地に二酸化炭素以外の炭素源を添加した光従属的な培養も可能である。

【0011】培養装置の材質としては、アクリル、ガラス、ポリカーボネートなど透明性が高く、細胞毒性の無いものならばいずれも可能であるが、屋外の使用を前提とする場合は、材料表面に紫外線吸収フィルムを適用することは有効である。装置の大きさは、培養槽内法の厚さが2cm以下であることが必要であるが、それ以外は培養に必要な容量に基づいて自由に高さと幅を設計できる。

【0012】また、培養槽内の温度を均一に制御できるのであれば、温度調節用の透明水槽のサイズも特に制限しない。前記のように光源も特に制限はなく、光強度も特に制限はない。通気量は、充分な攪拌効果を維持するために少なくとも1分間に1リッターの培養液あたり1リッター以上が望ましい。通気するガスは、空気に数%程度の二酸化炭素を添加した混合ガスが望ましい。気泡発生装置としては、ガラス製スパージャ等は不要で、

ステンレス製の内径5mm程度のチューブに1cmおきに1mm程度の穴を開けたもので充分に目的は達成できる。培養槽の上部は特に蓋をせず開放しておくことも出来るが、無菌的な培養を行う場合には蓋をして密封し、5-10%の過酸化水素を装置内に導入して滅菌することも可能である。

### 【0013】

【発明の実施の形態】以下、本発明による光合成微生物培養装置の好ましい実施の形態について図面を参照して説明する。図1は、本発明による光合成微生物培養装置の一例を示す分解斜視図であり、図において、1は培養槽本体、2は培養槽本体の温度を調節するための恒温水循環用透明ジャケットである。培養槽本体1は全体として薄い箱型状であり、両側面11、11は透明プラスチックあるいは透明ガラスなどの光透過可能な透明材料で作られている。そして、その対向する両側面11、11の内面間の距離（すなわち、培養槽の厚さ）は2cm以下とされている。培養槽本体1の一側部はやや幅広くされた膨出部12となっており、この膨出部を含めた培養槽本体1の上方は開放されている。

【0014】この実施形態において、培養槽本体1の下半分はジャケット2に収容されており、該ジャケット2には図示しない恒温水槽からの恒温水が、ジャケット2の底部近傍に設けた流入口21からジャケット2内に導入され、上方に設けた出口22から引き抜かれて、循環するようにされている。培養槽本体1の底部近傍には通気用チューブ3が設けてあり、該通気用チューブ3は培養槽本体1の底部に配置されている気泡発生用チューブ31（図2参照）に接続している。通気用チューブ3は図示しない送気ポンプに接続しており、送気ポンプを介して空気あるいは空気+二酸化炭素混合ガスが供給され、培養槽本体1内で気泡発生用チューブ31から気泡として培養液に放出される。

【0015】培養槽本体1の上方開放口13は、密閉用シリコンゴムシール4及び蓋5により密封的に閉鎖される。蓋5及び密閉用シリコンゴムシール4の培養槽本体1の膨出部12に相当する部分には、図示しない温度センサー、pHセンサー等の取付けや、酸アルカリ添加のための開口6が形成されており、また、培養槽本体1に相当する部分には空気抜き及びサンプリング用の開口8が形成されている。蓋5及び密閉用シリコンゴムシール4は適宜の締め具7により締め付けられ、密閉を確実にしている。なお、開放状態での培養を行う場合には、密閉用シリコンゴムシール4、蓋5、締め具7などは用いられない。

【0016】培養槽本体1の少なくとも側面位置には培養槽内の培養液に光を供給するための光供給手段が配置される。図1の例では、培養槽本体1の両側面11、11に近接した多数の蛍光灯群30が光供給手段とし配置されている。前記したように、光源は蛍光灯に限らず、

白熱電球、キセノンランプなどの人工光源を任意に用いることができ、また、自然の太陽光を利用してもよい。図示しないが、適宜の移動手段を用いて光源と培養槽の距離を調節可能とすることにより、多くの培養態様に適した光合成条件を作りだすことが可能となる。

【0017】図2は図1に示した培養槽の側面図であり、少し傾斜した湾曲邪魔板14が槽内に設置されている。湾曲邪魔板14は好ましくは槽底面から幾分浮き上がった状態とされており、両側面11、11の間に適宜の固定手段により固定的に配置される。この邪魔板は、培養槽の強度向上と、槽内の培養液の横方向への攪拌効率を上げpH及び温度制御の応答時間短縮を図る目的で設けられるものであり、実験的に、最適な傾斜角度、枚数などが設定される。なお、図2で矢印は、図示の形態に湾曲邪魔板14を配置し、ガス通気用チューブ3から気泡発生用チューブ31を通して培養槽内に空気を送った場合の培養液の流れの位置例を側面からスケッチしたものである。なお、図2では、蓋5に形成した開口6には温度センサーS1、pHセンサーS2が取り付けられている。

【0018】なお、図1、2に示したのは本発明による光合成微生物培養装置の一例にすぎず、多くの変形があり得る。例えば、気泡発生用チューブ31は装置の底部に一本しか示していないが、高さを変えて複数設置するようにしてもよい。また、通気用チューブ3を前記蓋5の膨出部分に設けた開口6を通して挿入するようにしてもよい。邪魔板の形状も図2に示したように湾曲していることは必須でなく、直線状のものであってもよい。また、培養槽本体1の厚さが2cm以下という制限を除けば、培養槽の形状、縦横の比も特に制限はない。

【0019】図3は、光合成微生物培養装置さらに他の実施形態であり、この例では、光源としての蛍光灯群30と培養槽とを交互に並置している。図において、41は二酸化炭素ポンベ、42は空気通気用コンプレッサーであり、二酸化炭素と空気とはガス混合器43により所望の混合比とされ、ガス通気用チューブ3を介して培養槽内に送り込まれる。また、図において、44は培養液回収用管路であり、該管路44を通して培養液を回収することができる。このように、複数の培養槽を並列的に配置することにより、容易に培養規模の拡大を図ることができる。

#### 【0020】

【実施例】以下、実施例により本発明を具体的に説明する。

(実施例1) 本発明の培養装置を用いて、海藻*Chlorococcum littorale*を培養した例を図4に示す。岩手県釜石湾より採取した海水と水道水を1対1で混合した1/2濃度の海水1リッターに対して以下の栄養塩類を添加したものを培養液として用いた。

#### 【0021】

<chem>KNO3</chem>	2.5	g
<chem>MgSO4 · 7H2O</chem>	1.25	g
<chem>KH2PO4</chem>	1.25	g
<chem>FeSO4 · 7H2O</chem>	2.2	mg
<chem>H3BO3</chem>	0.72	mg
<chem>MnSO4 · 7H2O</chem>	0.63	mg
<chem>ZnSO4 · 7H2O</chem>	0.056	mg
<chem>CuSO4 · 5H2O</chem>	0.020	mg
<chem>NaMoO4</chem>	0.0053	mg

【0022】この組成は、MC培地の硝酸塩の濃度のみ2倍にしたものである。この培地へ当該の微細藻を植え付け、2~3%の二酸化炭素を含んだ空気を、71/m inで、通気し攪拌した。温度は25°Cに保ち、pHは制御しなかった。乾燥重量は、適量の培養液を吸引ろ過してグラスファイバー製フィルター(Whatman, GF/C)上に細胞を集め、さらに3%のギ酸アンモニウムで洗浄した後、フィルターを105°Cで乾燥し、恒量に達した後に秤量して求めた。両側から24時間白色蛍光灯を連続照射し、平均光強度を1500 μmol/m²/sにした場合、1cm厚の培養槽で8.1g/dayを1/dの直線期増殖速度が得られた。この値は、これまでに、報告されている光独立栄養で培養された微細藻類の増殖速度の少なくとも2倍以上である。

【0023】培養槽の厚みのみを2cmと4cmに変え、他は同じ条件で培養を行った。その結果、図4に示す増殖曲線を得た。培養槽の厚さが4倍(4cm)になったときに直線期増殖速度が1/8になった。このことは、反応槽の厚さが小さければ小さいほど効率よく微細藻類を培養できることを示唆している。

【0024】すなわち、培養槽の厚みを4cm、2cm、1cmと薄くしていくと、図4に示すように、生産性(直線期増殖速度)が、1、4、8g/1/dと上昇している。培養液量は、4cm厚の場合を1とすると、2cmでは1/2、1cmでは1/4となる。光を受ける面積は一定であり、受光面積/液量は4cmの場合を1とすると、2、4となる。つまり、培養液の体積当たりに受ける光の量は2、4倍となる。従来の考えに従えば、生産性は受光量に比例するものであり、従って、生産性も2、4倍になると予測されたが、実際には、4、8倍となっており、これは予期できない結果であった。

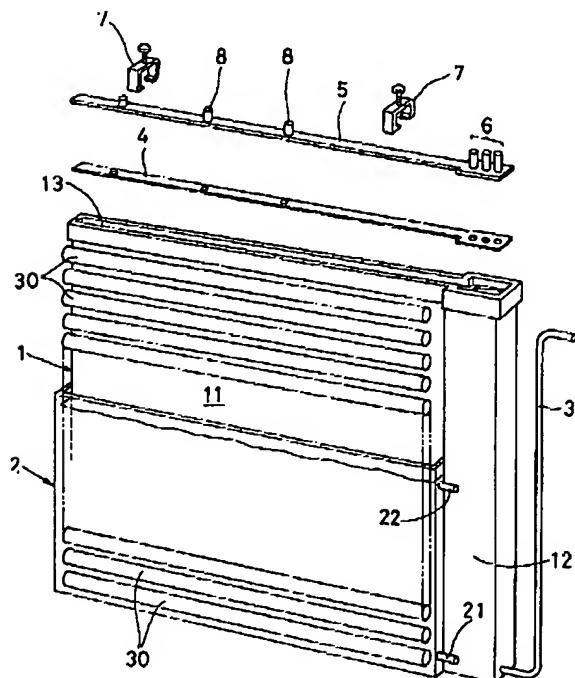
【0025】4→2cmでは、液量当たりの光エネルギーが2倍になっているだけなのに、生産性は4倍となっているのに対して、2→1cmでは、予想通り生産性は2倍となっている。つまり、4→2cmでの培養槽の厚さ減少の場合、単に厚さを減らしただけでは説明できない質的変化が起こっているといえ、本発明における、培養槽の両側面間の距離がほぼ2cm以下であることは、有意なものであることが立証されている。なお、1cmの培養槽で20時間目以降、増殖曲線の傾きが減少して

いるのは栄養塩（特に窒素源）の枯渇によるものと考えられる。

【0026】（実施例2）本装置を用いることによって、従来法では達成できなかつたほど高密度まで海産緑藻*Chlorococcum littorale*を培養した例を図5に示す。適当な時間間隔で細胞を遠心分離によって収穫し新鮮な培地に移して再び本装置で培養するという培地交換を行つた結果、最大細胞濃度は84 g dry cell/lに達した。

【0027】図中の矢印は培地交換を行つた時点を示す。この時光強度は2300  $\mu\text{mol}/\text{m}^2/\text{s}$ で、1 cm厚の培養槽を用いている。培地は、実施例1の培地の硝酸塩の濃度をさらに2倍（5 g/l）にしたものを使つた。クロレラ等を用いた従属栄養的培養（すなわち培地中に二酸化炭素以外の炭素源を添加した培養）で50~60 g dry cell/lと言う値が従来報告されているが、本発明の培養装置を用いることにより、また本発明の培養方法を用いることにより、さらに高濃度まで微細藻類を培養することが可能となつた。

【図1】



【0028】

【発明の効果】本発明は、光合成微生物の新規な培養装置と方法を提供するものである。光独立栄養微生物の培養は従属栄養微生物にコストと速度の面で劣ると考えられていたが、本発明の結果得られた光利用効率の向上と高密度培養の達成によって、光合成微生物の産業的利用という新たな局面を開拓することが可能となる。

## 【図面の簡単な説明】

【図1】本発明による光合成微生物培養装置の一実施形態を示す分解斜視図。

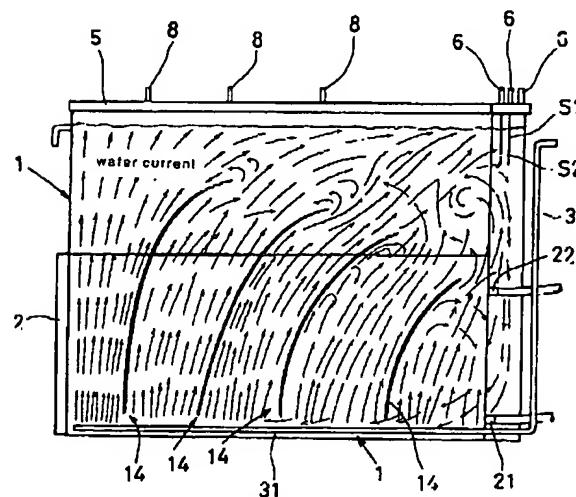
【図2】図1に示す装置の側面図。培養液の流れも示される。

【図3】本培養装置と光供給装置を並列に並べて培養規模を拡大した状態を説明する斜視図。

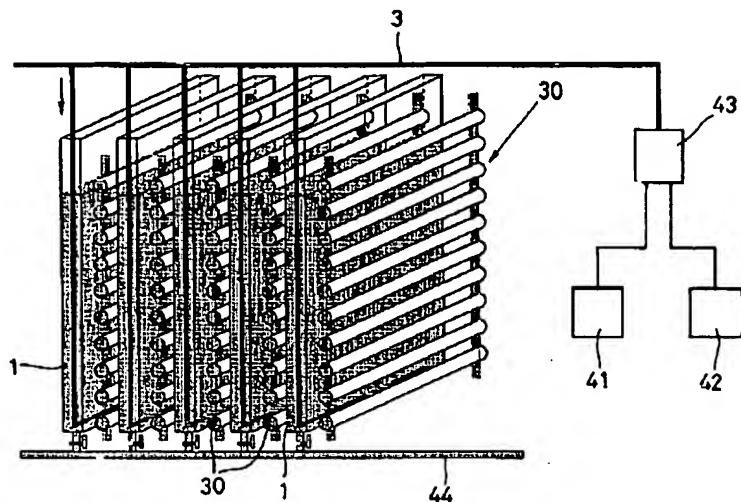
【図4】本培養装置を用いて海産緑藻*Chlorococcum littorale*を培養した結果を示すグラフ。

【図5】本培養装置を用いて、培地交換を繰り返して海産緑藻*Chlorococcum littorale*を高密度培養した結果を示すグラフ。

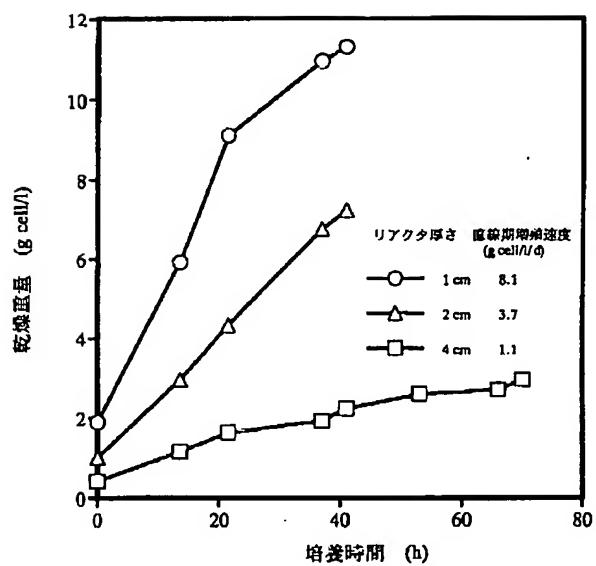
【図2】



【図3】



【図4】



【図5】

